44 PROPOFOL CONFIRMATION AND QUANTITATION BY GCMS

44.1 Summary

44.1.1 Biological samples are made neutral with sodium phosphate buffer (pH 7.0) and extracted with a mixture of pentane and diethyl ether. The extract is derivatized with BSTFA in the presence of trimethylammonium hydroxide and analyzed by GCMS for confirmation and quantitation by selected ion monitoring.

44.2 Specimen Requirements

44.2.1 1 mL of fluid(s) or 1 g of tissue(s) or comparable amounts of fluid or tissue dilutions/homogenates
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44.3 Reagents and Standards

44.3.1 Propofol

44.3.2 Thymol

44.3.3 0.1 M trimethylammonium hydroxide (TMAH)

44.3.4 Diethyl ether

44.3.5 Pentane

44.3.6 Acetonitrile

44.3.7 0.1 M disodium phosphate

44.3.8 0.1 M monosodium phosphate

44.3.9 Isopropyl alcohol

44.4 Solutions, Internal Standard, Calibrators, Controls

44.4.1 0.1 M Sodium phosphate buffer, pH 7.0: Mix 500 mL 0.1 M disodium phosphate and 250 mL 0.1 M monosodium phosphate and adjust pH as necessary.

44.4.2 Diethyl ether: pentane (2:1, v:v): Mix 100 mL diethyl ether with 50 mL pentane.

44.4.3 TMAH: Add 1.81 grams of trimethylammonium hydroxide pentahydrate to a 100 mL volumetric flask and qs to volum with isopropyl alcohol.

44.4.4 Propofol Stock Solution (1.0 mg/mL): Add 10 mg of propofol to a 10 mL volumetric flask and qs to volume with acetonitrile

44.4.5 Thymol Stock Solution (1.0 mg/mL): Add 10 mg of thymol to a 10 mL volumetric flask and qs to volume with acetonitrile.

44.4.6 Working Standard Solution A (0.1 mg/mL): Add 0.1 ml of a 1 mg/mL propofol stock solution to a 1.0 mL volumetric flask and qs to volume with acetonitrile.

44.4.7 Working Standard Solution B (0.01 mg/mL): Add 0.01 ml of a 1 mg/mL propofol stock solution to a 1.0 mL volumetric and qs to volume with acetonitrile.

44.4.8 Working internal standard (0.02 mg/mL): Add 0.02 ml of a 1 mg/mL thymol stock solution to a 1.0 volumetric flask and qs to volume with acetonitrile.

44.4.9 Blood calibrators, standards, and controls preparation:

44.4.9.1 To prepare the following calibration curve, pipet the following volumes of working standard solutions A or B into appropriately labeled 13 x 100 mm screw cap test tubes and then add 1 mL of whole blood.

- 2.0 mg/L Calibrator 20 µL of working standard solution A
- 1.0 mg/L Calibrator 100 µL of working standard solution B
- 0.4 mg/L Calibrator 40 µL of working standard solution B
- 0.1 mg/L Calibrator 10 µL of working standard solution B
- 0.08 mg/L Calibrator 8 µL of working standard solution B
- 0.04 mg/L Calibrator 4 µL of working standard solution B

44.4.9.2 Controls

9.4.9.2.1 Negative control. Blood bank blood (or comparable) determined not to contain propofol or thymol.

9.4.9.2.2 Positive control. In house control made from a different lot number, manufacturer, or aliquot.

44.5 Apparatus

44.5.1 Agilent GC/MSD, Chemstation software, compatible computer & printer

44.5.2 Test tubes, 13 x 100 mm round bottom, screw cap tubes, borosilicate glass with Teflon caps

44.5.3 Centrifuge capable of 2,000 – 3,000 rpm

44.5.4 Vortex mixer

44.5.5 Evaporator/concentrator

44.5.6 GC autosampler vials with inserts

44.5.7 Test tube rotator

44.5.8 GC/MSD parameters. Instrument conditions may be changed to permit improved performance.

44.5.8.1 Oven program.

- Equilibration time: 0.5 minutes
- Initial temp: 90° C
- Initial time: 2.00 minutes
- Ramp: 17° C/min
- Temp: 150° C
- Hold: 0.00
- Ramp: 30° C/min
- Final Temp: 290° C
- Hold: 2.00 minutes
- Run Time: 12.2 minutes

44.5.8.2 Inlet.

- Mode: Pulsed Splitless
- Temperature: 250° C
- Constant pressure: 11.83 psi
- Purge flow: 50.0 mL/min
- Total flow: 54.1 mL/min
- Injection volume: 2.0 µL

44.5.8.3 Column: HP-5 30 m x 0.25 mm x 0.25 µm

44.5.8.4 Detector Temperature: 280° C

44.5.8.5 Acquisition Mode: SIM

44.5.8.6 SIM ions:

propofol: 235, 236, 250

thymol: 207, 222

44.6 Procedure

44.6.1 Label clean 13 x 100 mm screw cap tubes accordingly, negative, calibrators, control(s) and case sample IDs.

44.6.2 Pipet 1 mL of blank blood, calibrators, controls and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes.

44.6.3 Add 20 µL of internal standard (thymol) working solution to each tube and vortex.

44.6.4 Add 1 mL 0.1 M sodium phosphate buffer (pH 7.0) to each tube.

44.6.5 Add 3 mL extraction solvent (diethyl ether:pentane) to each tube.

44.6.6 Cap and rotate tubes for 30 minutes.

44.6.7 Centrifuge at approx 2800 rpm for 15 minutes. Transfer organic (upper) layer to clean 5 mL conical bottom tubes.

44.6.8 Add 20 µL TMAH to each tube and evaporate to dryness under a stream of nitrogen at room temperature.

44.6.9 Add 100µL BSTFA to each tube, cap, vortex briefly, and heat at 85° C for 15 minutes.

44.6.10 Transfer small aliquot to appropriately labeled GC vials and inject 2 µl on GC-MSD.

44.7 Calculation

44.7.1 Calculate the concentrations by interpolation of a linear plot of the response curve based on peak height (or area) ratios versus calibrator concentration.

44.8 Quality Control And Reporting

44.8.1 See Toxicology Quality Guidelines

44.9 References

44.9.1 Stetson, P.L., Domino, E.F. and Sneyd, J.R. Determination of plasma propofol levels using gas chromatography-mass spectrometry with selected-ion monitoring. *Journal of Chromatography. 620* (**1993**) pp. 260-267.

44.9.2 Dwight Flammia, PhD. and Henry Bateman in house development.

♦**End**